Journal of Parasitology and Vector Biology

Volume 6 Number 7, July, 2014

ISSN 2141-2510

Academic Journals

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Table of Content: Volume 6 Number 7, July 2014

ARTICLES

Research Article

Assessment of insecticide treated bed net possession, proper utilization and the prevalence of malaria, in Dejen Woreda, East Gojam Zone, Ethiopia 92
Abeje Kassie, Melaku Wale and Tesfu Fekensa

Parasitic contamination on vegetables irrigated with Awash River in selected farms, Eastern Showa, Ethiopia 103
Girmaye Benti and Fekadu Gemechu

academicJournals

Vol. 6(7), pp. 92-102, July, 2014
DOI: 10.5897/JPVB2014.0147
Article Number: 71A38CA46275
ISSN 2141-2510

http://www.academicjournals.org/JPVB

Journal of Parasitology and Vector Biology

Full Length Research Paper

Assessment of insecticide treated bed net possession, proper utilization and the prevalence of malaria, in Dejen Woreda, East Gojam Zone, Ethiopia

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Received 20 January, 2014; Accepted 8 June, 2014

This study was conducted to assess the prevalence of *Plasmodium* species infection and proper utilization of insecticide treated bed nets in Dejen woreda, Amhara Regional State from October to December, 2011 to 2012. The study participants consisted of 403 people selected randomly from rural and Dejen town Kebeles of the Woreda. Examination for malaria parasites was carried out by using light microscope and rapid diagnostic test and a questionnaire administered to determine the knowledge, attitude and practice of study participants about insecticide-treated bed nets. The data collected was analyzed using χ^2 square test (for association of malaria) and descriptive statistics. Fifty (12.4%) of 403 study participants examined had malaria. Out of this, 25 (50%) were *Plasmodium vivax*, 22(44%) *Plasmodium falciparum* and 3 (6%) mixed infection of *P. falciparum* and *P. vivax*. The prevalence of malaria was significantly higher in rural Kebeles (13.7%) than in Dejen town Kebeles (6.7%) (χ^2 = 3.875, df = 1, P = 0.049). More males were infected compared to females (χ^2 = 7.842, df = 1, P = 0.005). The questionnaire based study showed that urban Kebeles had better knowledge, attitude and practice towards protection against malaria though people in rural Kebeles possessed more insecticide-treated bed nets than people in Dejen town (χ^2 =7.304, df = 1, p = 0.007). The findings of the present study have provided an empirical evidence for the need to implement effective malaria control measures to reduce malaria prevalence in Dejen Woreda.

Key words: Malaria, plasmodium, insecticide-treated bed nets (ITNs), prevalence, possession, utilization.

INTRODUCTION

Malaria is a disease caused by the protozoan parasites of the genus *Plasmodium*. The five species that commonly infect humans are: *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae* and *Plasmodium knowlesi* (World Health Organization (WHO) 2011). *P. falciparum* is found in the tropics and sub-tropics and it is the most important species as it is responsible for 50% of all morbidity and mortality from severe malaria. *P. vivax* is seen in tropics and sub-tropical areas and is less dangerous but more widespread. It is transmitted to humans by the bite of infected female *Anopheles* mosquito of more than 30

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species (WHO, 2011). In sub-Saharan Africa, *Anopheles gambiae*, *Anopheles arabiensis* and *Anopheles funestus* are the primary vectors of malaria parasites and show highly anthropophagic tendencies (Keating et al., 2004).

The disease is one of the world's most serious and complex public health problems. Its transmission is associated with topography, climate and socio-economic conditions (Kilian et al., 1999). The problem of the disease in Africa is aggravated by climate change (Solomon et al., 2011), poverty and lack of efficient controlling mechanisms (Solomon et al., 2011). It remains the leading cause of mortality and morbidity in sub-Saharan Africa (WHO, 2009), with 208 million cases and 863 thousand deaths reported in 2008 (Ngondi et al., 2011). An estimated 3.3 billion people were at risk of malaria in 2010 (WHO, 2011). Although of all geographical regions, populations living in sub-Saharan Africa have the highest risk of acquiring malaria. In 2010, 81% of cases and 91% of deaths were estimated to have occurred in the African region, with children under five years of age and pregnant women being most severely affected (Carter Center, 2011; WHO, 2011).

Africa experiences a complete spectrum of malaria epidemiology ranging from intense perennial transmission to unstable epidemic prone areas (MARA, 1998). According to RBM (2003), malaria accounted for up to 60% of all health facility visits in the Eastern African region. However, due to poor health care coverage and other factors, much of the malaria related illness and death actually occurs in the home, therefore, going unreported. The diseases epidemics affect non-immune populations in many highlands and semi-arid areas of the continents. It frequently affects high lands and semi-arid areas where populations lack immunity (Abeku, 2007). The control of malaria and its *Anopheline* vectors in Africa is less successful because of the occurrence of drug resistant parasites and insecticide resistant vectors, change in the resting behavior of mosquitoes (from indoor to outdoor) as a result of frequent indoor insecticide sprays, lack of efficient infrastructure, shortage of trained manpower, lack of equipment, financial constraints, lack of appropriate management and inability to integrate several methods of control (Toure, 2001; Howard et al., 2007). Therefore, we conducted the study to get accurate information not only to improve our knowledge of malaria epidemics, but also to assess the possession and proper use of insecticide-treated bed nets.

MATERIAL AND METHODS

Study design, area and population

A cross-sectional study was adopted in Dejen Woreda, East Gojam zone, Amhara Regional state of Ethiopia to assess the prevalence of malaria and proper utilization of insecticide treated bed nets. The study was carried out from October to December, 2011/2012. The area lies at altitudes of 1800 and 2880 m above sea level and the annual rainfall is 400 to 1000 mm. It is found between 10° 1' 12" and 10° 21' 16"N latitude and 38° 3' 3" and 38° 18' 30" E longitude. The study participants were drawn from Dejen Woreda rural and urban Kebeles, who visited Dejen Health Center from October to December, 2011/2012. About 403 individuals with various age and sex groups were examined for malaria test. Samples were selected by random sampling techniques from Dejen Health Center that came for treatment. Blood samples were collected and information gathered from documents. Concerned bodies were interviewed about the possession and proper utilization of insecticide treated bed nets in the area and demographic information were taken from patients selected with the help of questionnaire. In order to understand the knowledge, attitude and practices of population about the nature and mode of transmission of malaria and one of the preventive methods, insecticide treated bed net, a structured questionnaire was developed (Appendix 1). A total of 120 individuals were randomly selected whose age were greater than 15 from 403 individuals and the questionnaire was filled by the interviewer.

Sample size determination

Sample size was determined by using the formula (Daniel, 1999):

$$n=Z^2P\ (1-P)/d^2$$

Where n = sample size, Z = Z statistic for a level of confidence, P = expected prevalence or proportion, d = precision/marginal error.

The prevalence of malaria is not known in the study area. Therefore, P (expected prevalence) was taken as 50%. A minimum sample size of 384 generated using 5% marginal error.

Data collection and techniques

Blood film collection and testing

Data was collected from October to December. For blood film examination, the list of patients that visited Dejen Health Center was taken as the sampling frame. Blood film collection was carried out by experienced Dejen Health Center laboratory technicians by pricking the finger with disposable blood lancet. Peripheral blood smear examination of well prepared and well stained blood film is the gold standard in confirming the presence of malaria parasite (Payne, 1988). Thick and thin blood smears were taken on the same slide and identification numbers marked on the thin films. The thin films were fixed using 100% methanol and then all slides were stained with 3% Giemsa solution for 20 min. The staining technique and blood film examination were conducted according to a standard of World Health Organization protocols (Cheesbrough, 1987; Garcia, 2001). Then, parasite positivity was determined from thick smear and species identification was carried out from thin smear slide preparations. Examination for parasites was carried out by using light microscope and rapid diagnostic test in the Dejen Health Center by laboratory technicians. Rapid diagnositc tests (RDTs) for malaria are based principally on the detection of one of three antigens, histidine-rich protein-2 (HRP2), parasite lactate dehydrogenase (pLDH), and aldolase. *P. falciparum* was detected by Histidine-Rich Protein 2 (HRP-2). Parasite lactate dehydrogenase (pLDH) enzymes were used for the detection of *Plasmodium* species (pan-malaria), *P. falciparum* and *P. vivax.* The rapid diagnostic test was used in the event of electric power interruption in the Health Center. Malaria slides were stained with Giemsa and examined via high power ×100 oil immersion microscopy for the presence of malaria parasites.

Questionnaire

In order to understand the knowledge, attitude and practices (KAP) of the local population about malaria and insecticide treated bed nets, a structured KAP questionnaire was developed. The questionnaire was taken to 120 individuals selected randomly from 403 samples whose age was greater than 15. The questionnaire was originally developed in English and then translated in to Amharic. Direct observations were done on the possession and proper utilization of bed nets by the household members by the investigator.

Data analysis

Data collected on blood film examination and questionnaire surveys were analyzed using computer program SPSS v 16.0. Descriptive statistics was used to examine the characteristics of samples. Differences in prevalence of malaria between Dejen town and rural Kebeles and among ages and sexes were compared using Chi-square test. Results were considered to be statistically significant when at p-value of < 0.05.

RESULTS

ITNs distribution in the study area

The total number of households in the study area was 25,973. In 2009/10, a little over 40 thousand and in 2011/12 nearly 12 thousand bed nets were distributed. Each household had an average of 2 bed nets.

Socio-demographic characteristics and malaria prevalence

A total of 403 individuals (328 from rural Kebeles and 75 from urban Kebeles) participated in the study. Of these, 224 were males with age ranging from 1 to 78 years (mean 22.4) and 179 were females, age between 3 and 75 years (mean 23.5). Table 1 shows the socio-demographic characteristics of the study participants. The educational background of the study participants varied, ranging from illiterate to higher education. The majority (50.4%) of the participants were illiterate. In this study group 7.7% of the population were under 5 years, 14.1% were between 5 to 14 years and 78.2% participants were 15 and above years. Some 96.3% of the participants were Christian and 3.7% were Muslim. The majority of the participants were farmers (70.2%) and 4% were government employees.

Malaria positive individuals were identified from Dejen town and rural Kebeles, from October to December, 2012. Of the 403 blood films examined the overall malaria positivity was 50 (12.4%). There was no statistical significant variation between malaria infection and religion groups (Christian versus Muslim) ($\chi^2 = 0.472$, P = 0.492), age ($\chi^2 = 4.711$, P = 0.095). On the other hand, there was a statistical significant variation between residence, sexes, occupation and educational background of study participants (P < 0.05) as shown in Table 2. More males were examined for malaria than females. Some 16.5% of males and 7.3% of females examined were positive for malaria. Chi-square (χ^2) distribution test showed significant association with sexes (P < 0.05).

Majority of examined people were rural inhabitants. Significantly more people (13.7%) were infected in rural than in urban areas (6.7%) (P < 0.05). Regarding educational status, illiterate studied participants were highly (16.7%) infected with malaria, followed by those individuals found under the status of read and write (10.6%), elementary school (2.6%), high school and higher institution (0.0%), respectively. There was significant association between malaria parasite infection and educational status of the participants (P < 0.05). Concerning occupational status of studied participants, farmers were highly infected with malaria than others ($\chi^2 = 10.364$, P = 0.035).

The prevalence of *Plasmodium* species

The malaria species seen in the study group were *P. vivax* 25 (50%), *P. falciparum* 22 (44%) and mixed infection of *P. falciparum* and *P. vivax* 3 (6%). This showed that *P. vivax* species was the most prevalent malaria parasite in the Woreda. The mixed *Plasmodium* (*P. falciparum* and *P. vivax*) and *P. falciparum* species did not show any significant difference between sexes and residence. But, *P. vivax* prevalence was higher in males and varied markedly between females 5 (2.8%) and males 20 (8.9%) (P < 0.05) (Table 3).

In this study, the highest malaria prevalence shown in the 15 and above age-group is 14.3% compared to 6.5% in the under 5 years and 5.3% in the age group between 5 to 14 (Figure 1).

Knowledge, attitude and practice (KAP) study about mode of malaria prevention and insecticide-treated bed nets

Insecticide treated bed nets owner ship

Significantly more ITNs were owned by younger and middle aged people than older ones, rural people than towns and farmers than other occupations. Significantly less ITNs were owned by people who completed higher institution than other educational status (P < 0.05), but no significant difference between ITN ownership and sexes, and religion (P > 0.05) (Table 4).

Knowledge related to ITNs mechanism of action and utilization

Full awareness was reported about ITNs. Most owned it

Table 1. Socio-demographic characteristics of the study participants.

Variables	Study participants (n=403)	
	N	%
Sex		
Male	224	56.6
Female	179	44.4
Residence		
Rural	328	81.4
Urban	75	18.6
Religion		
Christian	388	96.3
Muslim	15	3.7
Occupational status		
Farmer	283	70.2
Government employee	16	4.0
Merchant	18	4.5
Daily labor	19	4.7
Others*	67	16.6
Educational level		
Illiterate	203	50.4
Read write only	141	35
Elementary school	38	9.4
High school	5	1.2
Higher institution	16	4.0
Age		
<5	31	7.7
5-14	57	14.1
≥15	315	78.2

*Stands for house wife, children, job seeking.

(95.8%) who had 2 ITNs (Table 5). Over half of ITNs were used by mothers and children (Figure 2). Only 59.1% of it was used properly. Nearly 60% of them were used all year round. There was strong belief that ITNs prevent malaria. ITNs were obtained for free. From the total 68 (59.1%) of properly utilized ITNs, urban Kebele individuals contributed 82.2% and rural Kebeles only 44.3 % (Figure 3).

Direct observation of ITNs used and owned in the study area

An attempt was made to assess proper usage and possession of mosquito net (ITN) in the rural and urban kebeles of Dejen Woreda. With regard to this, most of households in the urban Kebeles and some of rural households use mosquito net properly to protect themselves from mosquito bite. In most houses, there are ITN suspended over their beds. No one, in urban Kebeles was found using mosquito nets for other purposes. But, in rural Kebeles, even though each household has mosquito nets, only few households were observed using nets properly. During the study, many individuals in rural kebeles were observed using the mosquito nets for other purpose such as for rope (46.8%), cover of seedlings (29%), to hold different crops and house equipment in the market and other places (22%) and 2.2% for other

Table 2. Prevalence of malaria within specific socio-demographic characteristics (N=403).

Characteristics	Malaria infection			
	Positive n (%)	Negative n (%)	χ^2	p-value
Sex				
Male	37 (16.5)	187 (83.5)	7.842	0.005
Female	13 (7.3)	166 (92.7)		
Residence				
Rural	45 (13.7	283 (86.3)	3.875	0.049
Urban	5 (6.7)	70 (93.3)		
Religion				
Christian	49 (12.6)	339 (87.4)	0.472	0.492
Muslim	1 (6.7)	14 (93.3)		
Occupational status				
Farmer	43 (15.2)	240 (84.8)	10.364	0.035
Gov’t employee	0 (0)	16 (100)		
Merchant	2 (11.1)	16 (88.9)		
Daily labor	1(5.3)	18 (94.7)		
Others	4 (5.9)	63 (94.1)		
Educational level				
Illiterate	34 (16.7)	169 (83.3)	10.243	0.037
Read write only	15 (10.6)	126 (89.4)		
Elementary school	1 (2.6)	37 (97.4)		
High school	0 (0)	5 (100)		
Higher institution	0 (0)	16 (100)		
Age				
<5	2 (6.5)	29 (93.5)	4.711	0.095
5-14	3 (5.3)	54 (94.7)		
≥15	45 (14.3)	270 (85.7)		

different purposes. Normally, ITNs are used for protection against mosquitoes, but people highly value the fact that treated nets kill bedbugs. For some, that is an important motivating factor for using the net. But, this leads some to put the net directly on the mattress instead of hanging it over the bed. Figure 4.

DISCUSSION

Malaria remains to be one of the leading causes of illness and death in Dejen town and rural Kebeles. According to Dejen health office report, malaria was put in the third place next to helminthiasis and diarrhea from the top 10 leading diseases reported in 2011. The total prevalence of malaria in the present study was 12.4%. This shows that the malaria prevalence was high compared to the findings of the National Malaria Indicator Survey (4%) (Ministry of Health (MOH), 2007); from that of Estifanos et al. (2008) in Oromia and Southern Nations, Nationalities, and Peoples' Region (SNNPR) regions (2.4%), and that of Tekola et al. (2008) from Amhara Regional state (4.6%). However, this was closer to the prevalence (10.5%) among the population in South West Ethiopia (Amare et al., 2010). Environmental variation, sample size, nature of population and method diagnosis may contribute to the difference in different studies.

The high overall prevalence of malaria indicates that the burden of malaria is still high in different parts of the country in spite of the dramatic decrease in malaria

Table 3. Sex and residence specific prevalence of *Plasmodium* species.

Variables	No. examined	Presence of *Plasmodium* species		
		P. falciparum n (%)	*P. vivax* n (%)	Both *P. falciparum* and *P. vivax* n (%)
Sex				
Male	224	17 (7.6)	22 (9.8)	2 (0.9)
Female	179	8 (4.5)	6 (3.4)	1 (0.6)
χ^2		1.496	6.436	0.150
p-value		0.221	0.011	0.696
Residence				
Rural	328	23 (7)	25 (7.6)	3 (0.9)
Urban	75	2 (2.7)	3 (4)	0 (0)
χ^2		1.392	0.769	0.691
p-value		0.236	0.381	0.406

prevalence, the modeling on trends of health and health related indicators predicted over the last decade (Abraha and Nigatu, 2009). The local variation in malaria prevalence in Ethiopia is further complicated by the local variation documented in this study whereby the prevalence was significantly higher in the rural Kebeles compared to urban.

According to Hay et al. (2000), the peak transmission of malaria occurs following the main rainy season and a minor transmission peak occurs following light rainy season in the tropics. Therefore the relatively high peak transmission in October to December, 2011/2012, following the heavy rains, was to be expected in the study area. However, it was interesting to find that the prevalence of malaria in males was significantly higher than in females in October to December, 2011/2012. This finding might be explained by the fact that, in Dejen Woreda males spend the early part of the night working in their farms where they might be easily infected by exophagous mosquito bites, whereas most females do not have such risk as they normally are engaged in indoor household chores. The current study revealed that two species of *Plasmodium* (*P. falciparum* and *P. vivax*) that infect humans occurred in Dejen area. Previous studies indicate that four species are known in many places in Ethiopia (MOH, 2002) and five *Plasmodium* species in the world (WHO, 2011). So the diversity of *Plasmodium* species in this study area is low.

Federal Ministry of Health (FMOH) (2005) reported *P. falciparum* to be the dominant species during peak malaria transmission season while *P. vivax* tended to dominate during the dry season in Ethiopia. However, in the present study, *P. vivax* was slightly higher in prevalence than *P. falciparum* during the study period (October to December, 2011/2012), which is known to be within the peak transmission season. *P. vivax* prevalence was also higher in males than females. The reason could be, males are movable to different malarious parts of Ethiopia for daily labor and might be caught there and relapse when they came to this study area.

Concerned with educational level, in the current study illiterate individuals were more infected in malaria. This might be low awareness about the malaria control mechanisms and the improper hanging of ITN in their bed/other sleeping places. In contrast to the established convention that infection among children less than 5 years old in stable communities implies autochthonous malaria transmission (Giha et al., 2000), the finding in Dejen town and rural Kebeles, where the highest prevalence was in the age group 15 years and above, does not fit into the conventional characterization of malaria epidemiology based on age stratification. The higher prevalence in the age group 15 years and above may be explained by the inadequate coverage of household members with ITNs as each household received only 1.8 mean possession nets and most often only children slept inside the nets in majority of the cases, which leave the adults exposed to high risk of infection.

Comparing a net coverage among the rural and urban settings net possession, was found to be statistically significant among the residents. The proportion of ITN distribution per household was higher in rural Kebeles than in urban Kebeles and it is interesting to note that malaria prevalence determined in this study was inversely related to the intensity of ITN coverage. That is, the malaria infection is higher in rural than urban Kebeles. This could be the improper use of ITNs in the rural Kebeles and also observed that ITNs used for other purposes like rope and as a holding material. In the previous study, the proportion of ITN distribution per household was higher in urban Kebeles than in rural Kebeles (Berhane and Ahmed, 2008).

Table 4. Socio-demographic characteristics of respondents and possession of ITNs (n=120).

Characteristics	ITN ownership (N=120)		χ^2	p-value
	No	Yes		
	n (%)	n (%)		
Sex				
Female	4(7.7)	48(92.3)	2.857	0.091
Male	1(1.5)	67(98.5)		
Age				
16-30	0(0)	44(100)		
31-45	0(0)	51(100)	19.826	0.000
>45	5(20)	20(80)		
Residence				
Rural	0(0)	70(100)	7.304	0.007
Urban	5(10)	45(90)		
Religion				
Christian	5 (4.2)	113(95.8)	0.088	0.766
Muslim	0(0)	2(100)		
Occupational status				
Farmer	0(0)	70(100)		
Gov’t employee	5(33.3)	10(66.7)		
Merchant	0(0)	18(100)	36.522	0.000
Daily labor	0(0)	15(100)		
Others	0(0)	21(100)		
Educational level				
Illiterate	0(0)	35(100)		
Read write only	0(0)	59(100)		
Elementary school	0(0)	61(100)	36.52	0.000
High school	0(0)	5(100)		
Higher institution	5(33.3)	10(66.7)		
Malaria experience				
No	5(4.6)	104(95.4)	0.527	0.468
Yes	0(0)	11(100)		

In the present study area, majority of households had at least one ITN. A study conducted in Southern Nations, Nationalities, and Peoples' Region (SNNPR), Amhara and Oromia showed that only 5.3% respondents reported for the presence of at least one mosquito net in their household during the survey (Daddi et al., 2005). A study conducted in Kenya on community wide effect of Permethrin treated nets showed that control homes within 300 m of ITN villages received protection from ITNs in nearby homes (Net Mark, 2001). On the other hand, in this study area 100 and 90% of the households in the rural and urban Kebeles, respectively had at least one ITN. It was better than the 10% and less than 10% reported in the previous studies done in Ethiopia (WHO, 1993, 2005). The result from this study suggests that possession of bed net with mean number of 1.8 ITN per household was similar to the information (mean 1.8/household) gained from the other study done in

Table 5. Ownership of ITNs and conditions related to ITNs ownership.

Characteristics	Frequency (%)
Awareness of ITNs (n=120)	
Yes	100(100)
No	0 (0.0)
Ownership of ITN (n=120)	
Yes	115(95.8)
No	5(4.2)
Number of ITNs (n=115)	
One	14(12.2)
Two	101(87.8)
Utilizing of ITNs (n=115)	
Mother	13(11.3)
Mother &children	72(62.6)
Husband and wife	19(16.5)
All	11(9.6)
Proper utilization (n=115)	
No	47(40.9)
Yes	68(59.1)
Period of utilization (n=115)	
All year	67(58.3)
During rainy season	42(36.5)
During winter	6(5.2)
Sources of ITNs (n=115)	
Free	115(100)
With payment	0(0)
ITNs prevent malaria (n=120)	
Yes	117(97.5)
No	3(2.5)

Ghana (Sofonias, 2005) and almost the same as ITN distributed by Dejen Woreda health office (2 ITNs per household). Although the distribution of the net in the area has shown remarkable progress in the area, clearly, just increasing coverage will not be enough unless people use treated nets correctly and consistently.

In the present study concerned with utilization of ITNs most of the respondents replied mother and children slept under the ITNs and as mentioned before, malaria transmission is lower in under five years. This indicated that children who slept under ITN were less likely to be febrile during high transmission seasons (October to December). Previous studies have also documented the impact of long-lasting insecticide-treated bed nets (LLITN) on reduction of the burden of malaria. In Western Kenya where there is an intense transmission, 19% reduction in *P. falciparum* prevalence was observed due to LLITN (Terkuile et al., 2003).

The direct observation done for proper utilization of mosquito net (proper mounting) in the present study area showed that only 43.5% although self-report information was found to be over reported on direct observation.

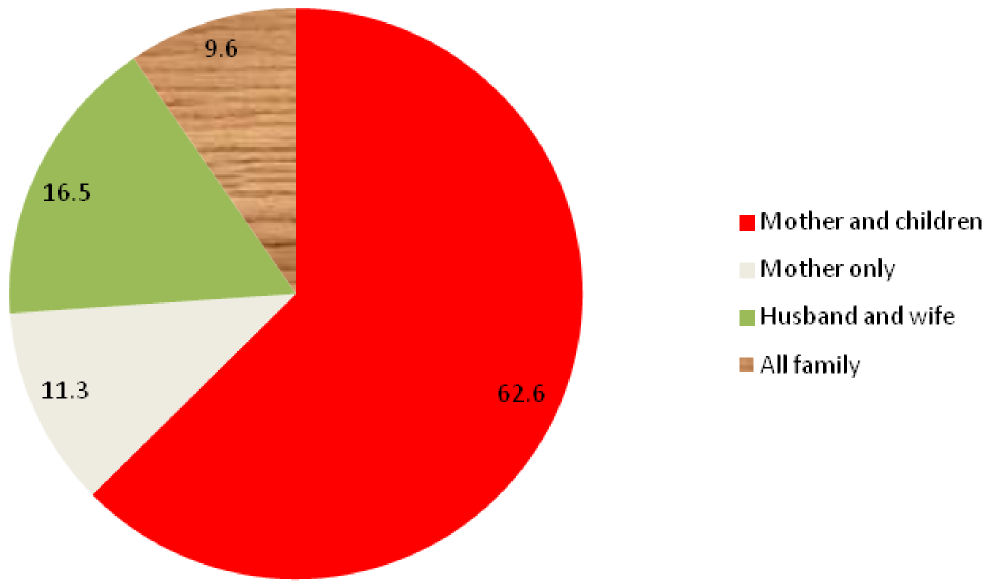

Figure 2. The proportional utilization of ITNs.

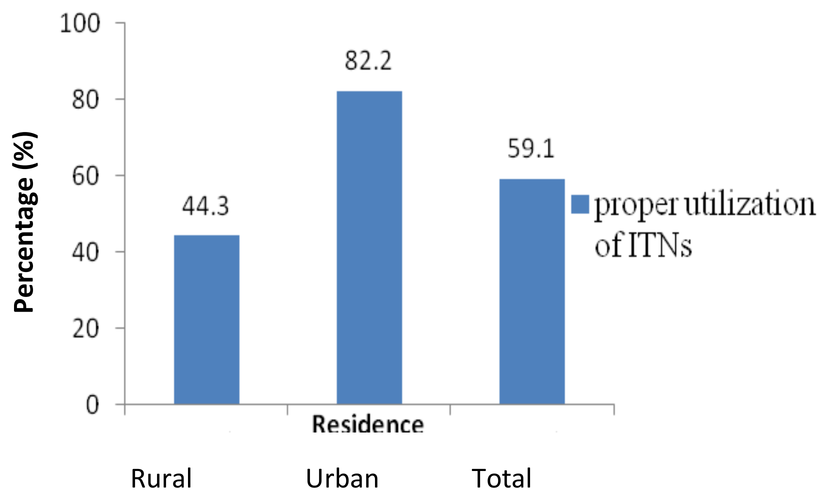

Figure 3. The proper utilization of ITNs by place of residence.

However this finding is encouraging compared to fewer than 10% reported from previous studies done nationally (MOH, 2002), but this was again lower than 69.9% gained from similar studies done in SNNPR, Ethiopia (Batisso et al., 2012).

In the current study, concerning the knowledge of the respondents about ITN, prevention mechanism had shown that majority of the respondents were able to identify insecticide-treated bed nets, prevent malaria infection and all of the respondents know the ITN. In the previous study, only Forty-one percent of the respondents had heard about the mosquito net (Daddi et al., 2005). In addition, Rhee et al. (2005) reported that, only 17% of those individuals stated using ITNs was an important method of prevention (Rhee *et al*., 2005). So awareness about ITN is high in the study areas. In this study only few responded that ITN do not prevent malaria. To aware all individuals health extension workers expected to do hard.

CONCLUSION AND RECOMMENDATIONS

The present study was an initial step to the understanding of malaria prevalence and possession and proper utilization of insecticide-treated bed nets in Dejen

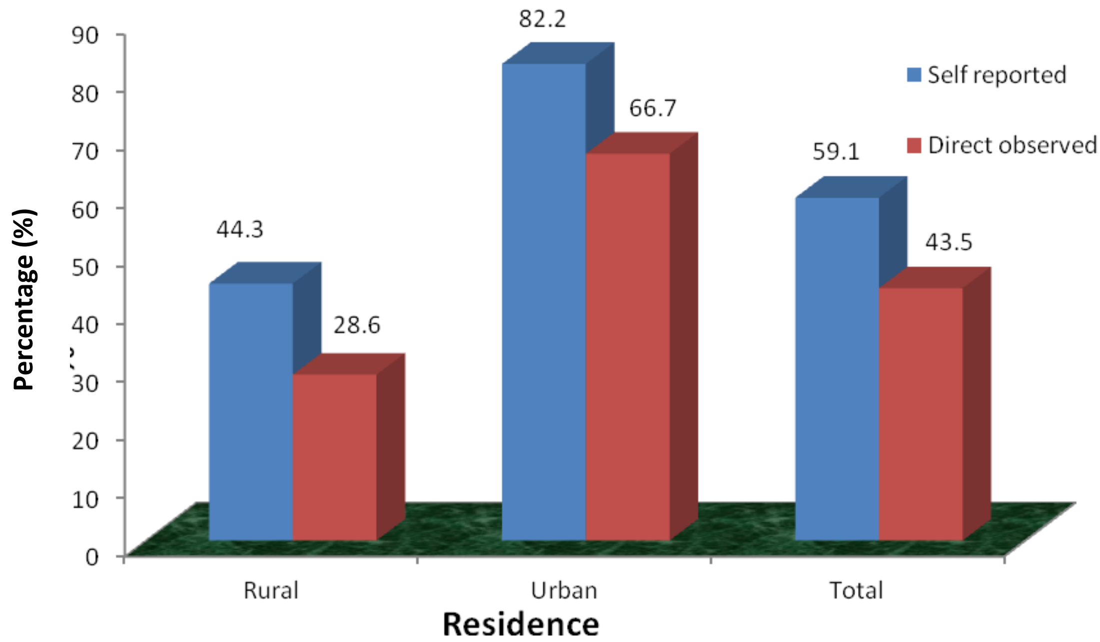

Figure 4. Relationship of self-reported ITN proper utilization among households with direct observation by place of residence.

town and rural Kebeles. Based on the finding of the study, *P. falciparum, P. vivax*and mixed infection of *P. falciparum* and *P. vivax* were the plasmodium species that caused malaria and *P. vivax* was higher in prevalence than *P. falciparum* in the study area. The possession and utilization of ITNs in the rural and urban Kebeles showed a promising result. Rural areas possessed more ITN than urban Kebeles. However, the prevalence of malaria was significantly higher in the rural Kebeles than urban Kebeles of Dejen Woreda due to improper use of insecticide-treated bed nets in the rural areas. Acceptability and willingness to use ITNs for malaria prevention was very high although the practice was low. Thus we recommend that communities should be strongly sensitized on the importance of ITNs for malaria prevention, and regular health education must be provided to raise individual and community awareness about the mode of malaria transmission, prevention and control, especially in the rural Kebeles.

Conflict Interests

The authors declare that there is no conflict of interests

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Appendix I

KAP questionnaire
Note that: The English version was translated in to Amharic.
Title: Assessment of the proper utilization of insecticide bed nets and the prevalence of malaria, in Dejen district, East Gojjam, Amhara region.

I. Area identification
1. woreda-------------
2. kebele--------------
3. House no. / ketena-------

II. Particulars of the study subjects
1. Name----------
2. Sex-------------
3. Age----------------------
4. Occupation-------------
5. Religion-----------------
6. Education:(literate, read&write only, elementary school, high school, higher institutions) (underline)
7. Do you know insecticide treated bed net? Yes/No (underline)
8. Do you have insecticide treated net in your home? Yes/No (underline)

8.1. If yes, how did you obtain the insecticide treated bed net? Free/with payment (underline)
8.2. How many ITNs do you have?
8.3. Do you use insecticide treated bed net properly?   Yes/No (underline)
8.4. Who usually sleeps under the net at night? Mother/children only/mother and children/all family (underline)
8.5. When do you sleep under the net? All year/only during rainy season/only during winter season (underline)
8.6. Does sleeping under a treated net reduce the risk of getting malaria? Yes/No (underline)
9. Have you ever experienced malaria or fever? Yes/No (underline)

academicJournals

Vol. 6(7), pp. 103-109, July, 2014
DOI: 10.5897/JPVB2014.0150
Article Number: 27A7D0146278
ISSN 2141-2510

http://www.academicjournals.org/JPVB

Journal of Parasitology and Vector Biology

Short Communication

Parasitic contamination on vegetables irrigated with Awash River in selected farms, Eastern Showa, Ethiopia

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Received 13 March, 2014; Accepted 24 June, 2104

This study was conducted from February, 2013 to April, 2013 to evaluate parasitic contamination of vegetable at Melka Hida and Wonji Gefersa wastewater irrigated vegetable farms that are found in Adama Woreda. A total of 72 vegetables samples were collected from both farms using a random sampling technique. Among all vegetables examined, *Ascaris lumbricoides* eggs were detected in 22.22% (16/72) of spinach (*Spinacea oleracea*), 16.67% (12/72) of lettuce (*Lactuca sativa*) and 23.61% (17/72) of cabbage (*Brassica oleracea* Linn). *Giardia intestinalis* cysts were detected in 18.06% (13/72) of spinach, 19.44% (14/72) of lettuce and 15.28% (11/72) cabbage samples. Of all parasitic contaminants *Entamoeba histolytica* cyst was the least detected parasite; 8.33% (6/72) of spinach, 12.5% (9/72) of lettuce and 8.33% (6/72) of cabbage samples from both farms. The high parasite contamination rates associated with these vegetable samples indicated poor farming practice employed in the overall production in the study area.

Key words: Awash River, contamination, parasite, vegetables.

INTRODUCTION

Vegetables are essential for good hearth, and they form a major component of human diet in every family. They are vital energy contributors that are depended upon by all levels of human as food supplement or nutrient (Duckworth et al., 1996). Food safety regardless of the specific food product should be a paramount concern to everyone, with parasites from contaminated vegetables being a potential health risk. According to Speer (1997), vegetables can become contaminated with enteric bacterial, viral and parasitic pathogens throughout the process of planting to consumption. The extent of contamination depends on several factors that include, among others, use of untreated wastewater and water supplies contaminated with sewage for irrigation (Amoah et al., 2007; Beuchat, 2002; Simões et al., 2001).

Intestinal parasites are common in fresh vegetables. Vegetables are reported to harbor intestinal parasites such as *Ascaris lumbricoides, Taenia* species, *Fasciola hepatica*, *Hymenolepis nana*, *Echinococcus* species, *Trichuris* species, *Enterobius vermicularis*,

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Trichostrongylus species, *Toxocara* species, *Strongyloides stercoralis, Giardia intestinalis*, *Entamoeba* species, *Iodamoeba butschlii*, *Blastocystis hominis* and *Cryptosporidium parvum* (Gharavi et al., 2002; Gupta et al., 2009; Abougrain et al., 2009; Uga et al., 2009). Intestinal parasitic infections are the top global health problems, whereas amoebiasis and ascariasis, are among the ten most common infections (WHO, 1987). Hence, consumption of raw vegetables plays an important role in the transmission of human parasitic infection (Amoah et al., 2005; Choi et al., 1982; Coelho et al., 2001; Daryani et al., 2008; Erdogrul and Sener, 2005).

Contamination of vegetables may occur through contacting with the soil, raw manure and sewage used as fertilizer on vegetable farms. In most cases, contamination of vegetables is associated with the water used for irrigation (Simoes et al., 2001). Use of sewage contaminated water for irrigation of vegetables is a common practice in developing countries. The access to clean water for irrigating vegetables is a major challenge. As an alternative, urban and peri-urban vegetable farmers in search of water for their crops have no other choice than to use water from these highly polluted sources. This raises public health concerns due to possible crop contamination with pathogens where vegetables are eaten uncooked (Amoah et al., 2006).

Unhygienic sewage disposal and absence of its treatment facilities pose potential health hazards through contaminating irrigated food crops with parasites in urban and suburban areas of African countries including Ethiopia (Damen et al., 2007; Nyarango et al., 2008; Srikanth and Naik, 2004; Weldesilassie et al., 2009). Many farm households in Eastern Showa that are irrigating their farmlands with wastewater are not aware of the risks or the potential harmful environmental consequences. This may be attributed to illiteracy, lack of adequate information and exposure to poor sanitary conditions for most of their lives. Several studies documented prevalence of intestinal parasites in different parts of Ethiopia including Oromia region through microscopic examination of stool samples collected from suspected human population (Legesse and Erko, 2004; Tadesse, 2005; Dejenie and Petros, 2009). However, research endeavors to detect parasites from environmental sources such as vegetables are scarce in Ethiopia (ROSA, 2009). Therefore, the aim of this study was to assess the degree of parasitic contaminations on selected vegetables irrigated with Awash River at Melka Hida and Wonji Gefersa farms.

MATERIALS AND METHODS

Description of the study area

This study was conducted at Melka Hida and Wonji Gefersa wastewater irrigated vegetable farms that are found in Adama Woreda. Wonji Gefersa is located in East Showa zone, Oromia region, 107 km away from Addis Ababa. It is located at a latitude of 8° 26' 59'' North and longitude of 39° 16' 48'' East. It has an elevation of 1588 masl and its temperature and rain fall is 23°C and 500 to 800 mm, respectively (Environmental Protection Authority, 2005). While Melka Hida is found in Adama town administrative zone, Oromia region, 99 km away from Addis Ababa. It is located at latitude of 8° 33′ 0″ North and longitude 39° 16′ 12″ East. It has an elevation of 1620 masl (Environmental Protection Authority, 2005) (Figure 1).

Study Design

Preliminary surveillance study was conducted before starting the test experiment at Melka Hida and Wonji Gefersa vegetable farms using Awash River for irrigation. Accordingly 160 sample respondents were purposively selected and provided with already prepared questionnaire. The questions were interpreted for few producer respondents who can't understand the matter. Out of the selected producer 135 were males and 25 were females, all of which are taken as literates. According to the answer obtained and analyzed from the respondents most of the problems that frequently faced them were recognized by the professional physicians and so related with the parasitic contaminations from the vegetables and fruits. Based on these the following three parasitic contaminants namely giant roundworm (*A. lumbricoides),* Giardia *(Giardia intestinalis) and* parasitic protozoan *(Entamoeba histolytica*) have seen to be the most dominant and leading parasite diagnosed. Then, a cross sectional survey test experiment was conducted to assess contamination of specific parasites on the main leafy vegetables [lettuce (*Lactuca sativa*), cabbage (*Brassica oleracea* Linn), and spinach (*Spinacea oleracea*)] that were grown in Melka Hida and Wonji Gefersa vegetable farms. The samples were regularly collected three times at three week interval from February, 2013 to April, 2013.

Sample collection

A total of 72 samples comprising three types of fresh vegetables (cabbage, lettuce and spinach) were collected from Melka Hida and Wonji Gefersa vegetable farms using a random sampling technique. Recently, mature leaves of lettuce, cabbage and spinach were sampled at early maturity according to methods used by Itanna (1998). All samples were collected aseptically in a sterilized universal container and plastic bags and transported to Dilla University using a cooler box for laboratory processing. Analysis was conducted within 24 h of arrival at the Parasitology Laboratory of Dilla University.

Parasitological analyses of vegetables

In the laboratory, 100 g of each fresh vegetable sample was chopped into small pieces and put into a clean beaker containing enough physiological saline solution (0.85% NaCl), to wash the sample. After removing fragments of the vegetable sample from the washing saline using clean forceps, it was kept about 24 h for sedimentation to take place. After 24 h sedimentation, the top layer of the washing saline was carefully discarded leaving 5 ml of the sediment. This was finally centrifuged at 2000 rotations/min for 5 min by HERMLE Z200A centrifuge. After discarding the supernatant, the residue was mounted on slides, stained with Lugol's iodine solution and examined under the compound light microscope to examine the samples for intestinal parasites: *A. lumbricoides* eggs, *E. histolytica* cysts and *G. intestinalis* cysts (Abougrain et al., 2009; Uga et al., 2009). To increase the chance of parasite detection, three slides were prepared for each vegetable sample.

Data analysis

For data analyses, descriptive statistics in percentage was computed for the pre surveillance data and parasitic contamination of vegetable samples. Chi-square test was used for comparison between vegetable contamination rates using SPSS version 16.0. A P-value ≤0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The study results of the pre surveillance and test experiment data on parasitological analysis of leafy vegetables were explained as shown in Tables 1 and 2, consecutively.

The aforementioned results (Table 1) show that 67.5% (108) of the respondents said that their income depends on the vegetable production. Producers also asked for why and which types of vegetables they produce and so, 50% (80) and 64.4% (103) explained that they were produced for the purpose of income generation and vegetable types like cabbage, lettuce and spinach, respectively. Similarly, 87.5% (140) of the producer said that the disease agents are caused by different parasitic worms and other pathogenic microbes. Among the parasites *A. lumbricoides, E. histolytica* and *G. intestinalis* are the predominantly mentioned by respondent.

Based on the information obtained from the surveillance, regular experiment was conducted to test for the presence of the aforementioned parasite. The results (Table 2) showed that cabbage samples collected from both Melka Hida and Wonji Gefersa farms, 17 (23.61%) were found to be positive for *A. lumbricoides* eggs. In developing countries, intestinal parasites are very common. Fresh vegetables are an important route of their transmission. Daryani et al. (2008) reported the detection of intestinal parasites in 29% (13/45) of native garden vegetables. The distribution of parasites on vegetables between Melka Hida and Wonji Gefersa was significantly different (χ^2=3.772, P=0.042). Variation may be due to different sections of the wastewater drainage canal in Wonji Gefersa farm which receive different load of faecal contamination.

A. lumbricoides eggs were the predominant intestinal parasite in this work. These include 22.22% (16/72) in spinach, 16.67% (12/72) in lettuce and 23.61% (17/72) in cabbage. The results showed that cabbage is relatively highly contaminated and lettuce is the least contaminated (Table 2). This might be due to cabbage nearby the soil as compared to the other vegetables that was directly contaminated with irrigation water or indirectly by contact with contaminated soil, may aggravate the rate of contamination. Other studies show that vegetables with dense foliage were most contaminated than those growing on surface (Idrissa et al., 2010). The dense foliage would protect the helminth eggs against unfavourable conditions to their survival and persistence, such as sunlight, drying, and wind (Dssouli 2001; Dssouli et al., 2006).

In relation to this finding, Abougrain et al. (2009) reported on other vegetables that helminth eggs and *G. intestinalis* cysts were detected in 58% (73/126) of vegetables examined. These include 14% (5/36) of tomato, 42% (15/36) of cucumber, 96% (26/27) of lettuce and 100% (27/27) of cress samples. This shows that the use of sewage water plays important role in the transmission of parasitic disease to human through consumption of such vegetables (Gupta et al., 2009). *A. lumbricoides* eggs were detected from lettuce with a value of 25% (9/36) from Melka Hida and 8.33% (3/36) from Wonji Gefersa. There was significant variation between the sites (χ^2=3.600, P=0.050), Melka Hida having the highest rate. This may be due to contamination during production and application of human excreta, animal manure as a fertilizer. The results of this study is comparable with the previous finding that showed Chinese cabbage (*Brassica pekinensis*) had the highest degree of contamination (91.1%) by *A. lumbricoides* eggs (Choi and Lee, 1992).

G. intestinalis cysts were detected in 18.06% (13/72) of spinach, 19.44% (14/72) of lettuce and 15.28% (11/72) of cabbage samples from both farms. There was also significant difference in the prevalence of parasites stages (χ^2 = 5.675, P = 0.017). This indicates that the prevalence of *G. intestinalis* cyst was more prevalent on lettuce collected from Wonji Gefersa farm than the other vegetables. This may be due to the production related sources of parasitic contaminants associated with practices of using untreated wastewater for irrigation.

On the other hand, the prevalence of *G. intestinalis* cyst examined on cabbage and spinach from both farms were not significantly different (χ^2 = 2.683, P = 0.101; χ^2 = 2.347, P = 0.126), respectively (Table 2). The finding of this study is in line with Daryani et al. (2008) who reported detection of intestinal parasites in 29% (13/45) of native garden vegetables consumed in Ardabil city, Iran. Similarly, Abougrain et al. (2009) examined 126 samples of four different types of fresh salad vegetables from wholesale and retail markets in Tripoli, Libya of which 58% were positive for helminth eggs and *G. intestinalis* cysts. The variation in the prevalence might be due to sample size difference.

Moreover, *E. histolytica* cyst was detected in 8.33% (6/72) of spinach, 12.5% (9/72) of lettuce and 8.33% (6/72) of cabbage samples from both farms. The results of the study indicated that the prevalence of *E. histolytica* cyst was the least. However, there was significant difference (χ^2 = 3.175, P = 0.045). The result of the study revealed that lettuce collected from Wonji Gefresa was more contaminated by *E. histolytica* cysts. As shown in Table 2, *E. histolytica* cysts was not significantly different (χ^2 = 2.683, P = 0.101; χ^2 = 2.347, P = 0.126) between cabbage and spinach, respectively.

In connection with this finding, Damen et al. (2007)

Table 1. The percentage and frequency of pre surveillance data collected.

Parameter	Frequency	%
Sex		
Male	135	84.4
Female	25	15.6
What is your source of income?		
Vegetables production	108	67.5
Other crops production	15	9.4
Both	37	23.1
Which type of vegetables do you cultivate?		
Cabbage	8	5.0
Lettuce	10	6.2
Spinach	12	7.5
Beetroot	4	2.5
Tomato	23	14.4
Cabbage, lettuce and spinach	103	64.4
Purpose of production		
Income generation	80	50
Home consumption	24	15.0
Both	56	35.0
Source of water for the production of vegetables		
Awash River	153	95.6
Under ground water	7	4.4
Would you think this water (Awash River) is contaminated?		
Yes	136	85
No	24	15
If you say yes for above question, what is the possible sources for contamination		
Wastewater from the municipality directly join Awash River	34	21.2
Community nearby Awash River defecated on open space	47	29.4
Both are the source of contamination of Awash River	79	49.4
Did you get sick in last one year?		
Yes	115	71.9
No	45	28.1
What is the possible source for disease occurrence?		
Consumption of improperly cooked vegetables	110	68.8
Personal hygiene	28	17.5
Utensil used	22	13.2
Which types of disease causing agents are identified in laboratory diagnosis?		
Parasite	140	87.5
Bacteria	20	12.5
If you say parasite for above question, which types of the parasites predominantly observed?		
Ascaris lumbricoides	40	25
Entomoeba histolytica	20	12.5

Table 1 cont'd

Giardia intestinalis	15	9.4
Fasciola hepatica	4	2.5
Strongyloides stercoralis	10	6.2
Cryptosporidium parvum	6	3.8
Ascaris lumbricoides, Entomoeba histolytica and *Giardia intestinalis*	65	40.6
What types of recommendation recommended by the physician about the quality of food consumptions?		
Not to eat improperly cooked vegetables	90	56.2
Wash the vegetables properly with clean water before consumption	50	31.2
Treat the water used for vegetables production	20	12.5

Table 2. Prevalence of three purposively selected parasites from cabbage, lettuce, and spinach samples of both farms (Melka Hida and Wonji Gefersa).

Vegetable type	No. of examined sample	Detected organisms	Site					χ^2 test	P-value
			MH	%	WG	%	Total (%)		
Cabbage	12	*A. lumbricoides* eggs	5	13.89	12	33.33	23.61	3.772	0.042
		G. intestinalis cyst	3	8.33	8	22.22	15.28	2.683	0.101
		E. histolytica cyst	1	2.78	5	13.89	8.33	2.909	0.088
Lettuce	12	*A. lumbricoides* eggs	9	25.0	3	19.44	16.67	3.600	0.050
		G. intestinalis cyst	3	8.33	11	30.56	19.44	5.675	0.017
		E. histolytica cyst	2	5.56	7	19.44	12.50	3.175	0.045
Spinach	12	*A. lumbricoides* eggs	6	16.67	10	27.78	22.22	1.286	0.257
		G. intestinalis cyst	4	11.1	9	25.00	18.06	2.347	0.126
		E. histolytica cyst	2	5.56	4	11.11	8.33	0.727	0.394

MH: Melka Hida, WG: Wonji Gefersa.

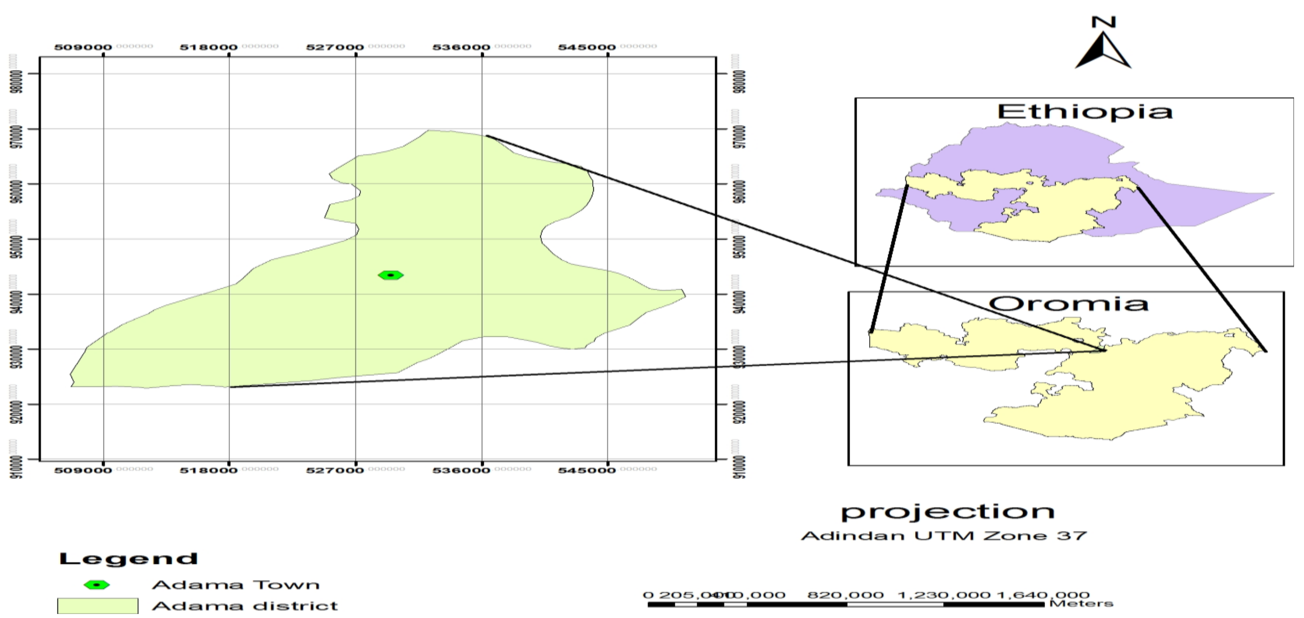

Figure 1. Location of the study area (Aynalem, 2010).

isolated ova of *A. lumbricoides* eggs (17.1%) and *E. histolytica* (14%) from different vegetables in Jos, Nigeria. In general, the study showed significant differences in the occurrence of pathogenic intestinal parasites detected from fresh vegetables. Several factors may include geographical location, type and number of samples examined, methods used for detection of the intestinal parasites, type of water used for irrigation, and per harvesting handling methods of such vegetables may contribute to such differences. According to Bethony et al. (2006) environmental factors play a great role in the incidence of intestinal parasitic infection as hot and humid tropical climate favour increased parasite prevalence. Based on variation in climatic and geographic zones in Ethiopia, it should be evident that there are macro and micro environmental factors contributing to the differences in prevalence of intestinal parasites (Jemaneh, 2000).

Conclusions

This study showed that *A. lumbricoides* eggs, *G. intestinalis* cysts and *E. histolytica* cyst were recorded on vegetable samples of both farms. Taking account of results obtained, it is clear that irrigation process of these vegetables fields has been performed using contaminated water sources (wastewater). Therefore, great attention should be given in using contaminated water for production of vegetables for the public health perspective. An adequate treatment of the sewage water and banning wastewater use for irrigation of plants intended for human consumption, among others, should be implemented. Health promotion and education on the mode of transmission of disease, environmental sanitation and eating habits will enhance the prospect for the control of parasitic infections.

ACKNOWLEDGEMENTS

The authors thank Dilla University College of Natural and Computational Science for the financial, material support and laboratory facilities provision. They also appreciate the efforts of Wonji Gefersa Administrative for helping in sample collection.

Conflict Interests

The authors declare that there is no conflict of interests regarding the publication of this article.

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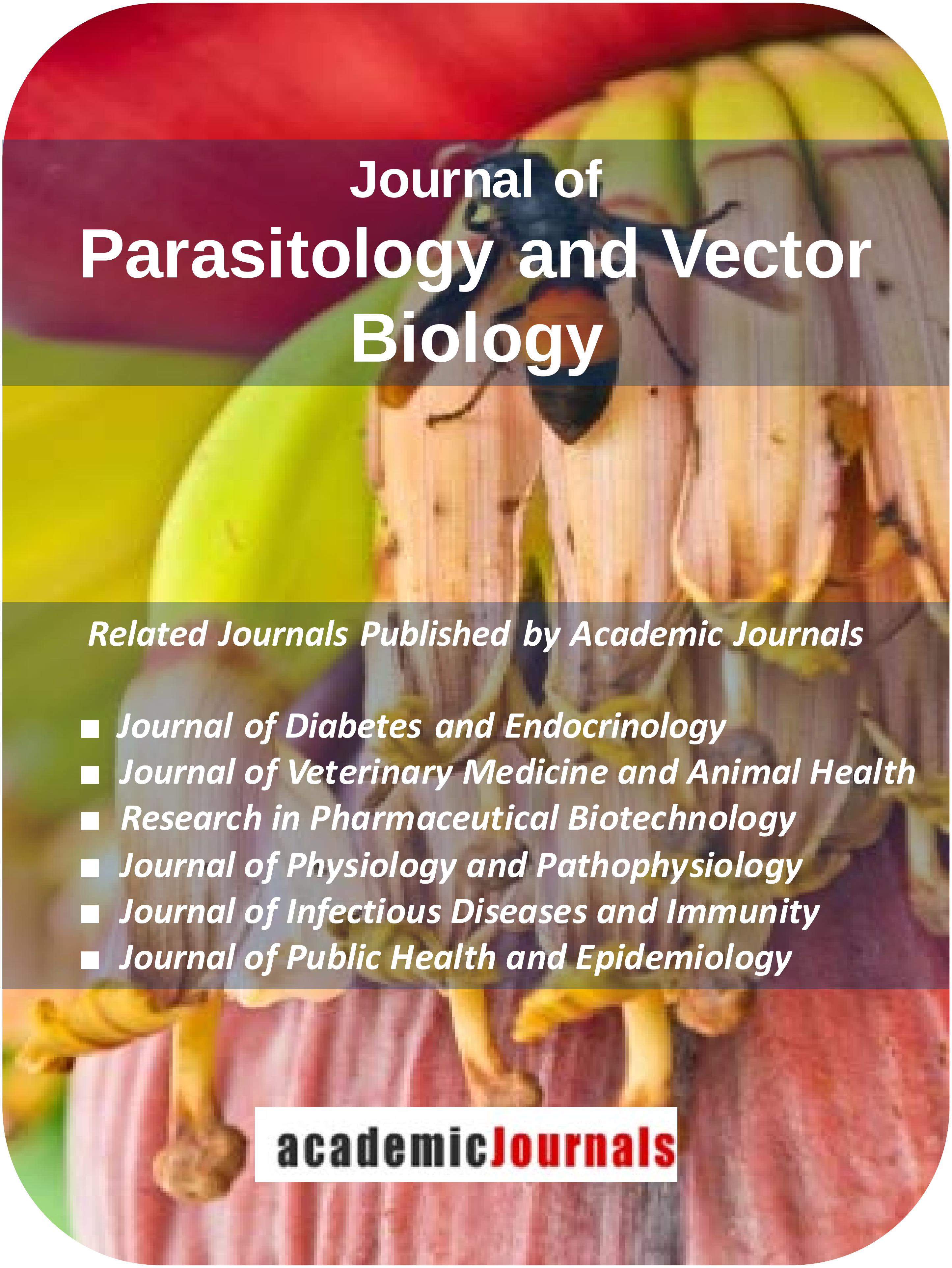

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